Quantitative analysis of spinally projecting adrenaline-synthesising neurons of C1, C2 and C3 groups in rat medulla oblongata

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Abstract

Spinal projections of the phenylethanolamine N-methyltransferase (PNMT) immunoreactive neurons of the medulla were investigated using a combination of immunohistochemistry and retrograde transport of colloidal gold particles conjugated to cholera toxin B subunit (CTB-gold). The PNMT-containing adrenergic neurons were localised in three groups, the C1 group in the rostral ventrolateral medulla, the C2 group in the nucleus tractus solitarius/dorsal vagal motor complex in the dorsal medulla and the C3 group in the mediodorsal medulla. The C1 group contained 72% of the medullary PNMT-IR neurons, while C2 comprised 13% and C3 15% of the medullary PNMT-IR neuron population. CTB-gold was injected in the area of the intermediolateral cell column in either upper (T2–T4) or lower (T8–T9) thoracic spinal cord and retrogradely labelled cells were found in the areas of the C1, C2 and C3 groups and in other regions of the medulla which did not contain PNMT-IR neurons. After tracer injections bilaterally at levels T2–T4, retrograde labelling suggested that at least 21% of all medullary PNMT-IR neurons projected to these levels. As a proportion of each group, 26% of C1, 9% of C2 and 33% of C3 neurons projected spinally to T2–T4. After tracer injections bilaterally at levels T8–T9, retrograde labelling suggested that at least 17% of all medullary PNMT-IR neurons projected to these levels. As a proportion of each group, 16% of C1, 9% of C2 and 30% of C3 neurons projected spinally to T8–T9. These figures represent minimum numbers since it is impossible to ensure that every neuron has equal access to the tracer. The results demonstrate that contrary to previous belief, the PNMT-IR innervation of the spinal cord derives from PNMT-IR neurons in the dorsal medulla, as well as from the rostral ventrolateral medulla. Indeed 24% of the PNMT-IR neurons terminating at spinal cord levels T2–T4, and 35% of those terminating at levels T8–T9, derive from the dorsal (C2 and C3) medullary cell groups. Since the PNMT-IR projections are directed towards the intermediolateral cell column, it seems likely that all three groups of medullary adrenaline-containing neurons contribute to the regulation of sympathetic outflow.

Introduction

The enzyme phenylethanolamine N-methyltransferase (PNMT) converts noradrenaline to adrenaline and is considered to be a marker for neurons that use adrenaline as a transmitter. PNMT-immunoreactive (PNMT-IR) neurons have been demonstrated in brain of several species, including rat [5,29,32], cat [21], monkey [12] and man [23,36]. It is in the rat that the PNMT-IR neuronal system has been studied most extensively. Adrenaline cell groups in the brainstem have been localised in three areas, the C1, C2 and C3 groups [30,32]. Although other neurons displaying
PNMT-IR have been localised in the hypothalamus, these neurons do not contain the other enzymes of catecholamine synthesis [19,51] and are not generally believed to contain adrenaline. The rostral ventrolateral medulla (RVLM), the area of the C1 adrenaline neurons, plays an important role in maintaining vasomotor tone and mediating vasomotor reflexes, probably via the direct projections of the RVLM neurons to the intermediolateral cell column of the thoracic spinal cord. Although the C1 PNMT-IR neurons of the RVLM project to this area of spinal cord [49,50], the vasomotor functions of the RVLM cannot be attributed solely to these PNMT-IR neurons, since a variety of other putative neurotransmitters have also been localised in spinally projecting neurons of the RVLM. These include neuropeptide Y (NPY; [10]), serotonin, substance P and thyrotropin-releasing factor, in some neurons localised together [33], enkephalin [31,41] and the enzyme of GABA synthesis, glutamic acid decarboxylase [42]. Studies have supported the hypothesis that the C1 adrenaline neurons of the RVLM play a major role in the regulation of sympathetic tone and blood pressure. Stimulation of the C1 area of the RVLM increases sympathetic activity and blood pressure [16,21,40,44,48,50,52,61], and electrophysiological studies have suggested that a population of spinally projecting, barosensitive neurons in RVLM [11,40,58] are likely to contain PNMT [26,45].

The first observations in the rat suggested that only the adrenaline-containing neurons of the RVLM C1 group projected to the spinal cord [49]. This was supported by experiments in cat [21] and monkey [12]. More recently some PNMT-IR neurons of the dorsal medulla have been observed to be labelled retrogradely from the spinal cord [25,39,46,54], but a detailed study of this dorsal bulbospinal projection has not been made. Since these dorsal medullary PNMT-IR neurons, together with the ventrally located PNMT-IR neurons, might contribute to the regulation of sympathetic activity and blood pressure via a direct spinal projection, it is important that their location be precisely defined as an aid to further investigation. It was the aim therefore of the present study to map and quantitate the adrenaline-containing neurons that project to the spinal cord from both the dorsal and the ventral medulla. We have used cholera toxin B subunit conjugated to 7-nm colloidal gold particles (CTB-gold) to retrogradely label spinally projecting neurons in the medulla in rats. In combination with PNMT immunohistochemistry we have demonstrated that PNMT-containing neurons belonging to all three medullary adrenaline groups, that is C1, C2 and C3, project to the intermediolateral cell column area of the thoracic spinal cord.

Materials and Methods

Surgical procedures

Rats (Wistar Kyoto, male, 250–350 g) were anaesthetised with a sodium pentobarbital (Nembutal, 30 mg/kg)/chloral hydrate (100 mg/kg) mix i.p. After femoral artery cannulation (polyethylene SP10 tubing), rats were intubated, and placed in a stereotaxic holder (David Kopf). Short-lasting ganglionic blockade was induced by suxamethonium chloride (4 mg/kg i.a., Anectine, Wellcome) and rats were artificially ventilated (Harvard 608 ventilator). By dorsal incision the spinal column was exposed in either upper thoracic (T2–T4) or lower thoracic (T8–T9) levels. Partial laminectomy exposed the dorsal surface of the spinal cord. A glass pipette (Clay Adams), outside tip diameter 30–50 μm, was placed in the spinal cord to lie in the area of the intermediolateral cell column (0.6 mm lateral to midline, 0.7–0.9 mm below dorsal surface). The pipette was filled with cholera toxin B subunit conjugated colloidal gold (CTB-gold [38]; now commercially available from Gilt Products, Flinders University Department of Medicine, Bedford Park, Australia). GTB-gold (250 nl) was injected over 10 min (25-nl injections each 60 s). The pipette was withdrawn and placed further along the cord using the same coordinates. In total 1 μl of CTB-gold was injected into each rat, either bilaterally (2×250 nl each side of spinal cord) or unilaterally (4×250 nl on one side of spinal cord). The CTB-gold injections were placed over a 2-mm section of spinal cord. After CTB-gold injection the exposed cord was covered with Gelfoam (Upjohn), the overlying muscle and skin was sutured, and the arterial cannula was
withdrawn. With resumption of spontaneous respiration rats were extubated. 6–10 days after CTB-gold injections rats were anaesthetised (hexobarbitone sodium, Brietal, 40 mg/kg i.p.) and injected with colchicine (100 µg in 10 µl 0.9% saline intracereally). At 48 h after colchicine rats were deeply anaesthetised with sodium pentobarbital (60 mg/kg) and perfused transcardially with 150 ml 0.1 M phosphate buffered saline pH 7.2, followed by 1 l fixative, formaldehyde (4%), glutaraldehyde (0.05%) and picric acid (about 0.2%) in phosphate buffer 0.1 M, pH 7.2 [56]. Brain and spinal cord were post-fixed at 4°C overnight.

**Immunohistochemical procedures**

Transverse 50-µm Vibratome (Oxford Instruments) sections, cut perpendicular to the ventral surface of the brainstem, were taken from the level of the pyramidal decussation caudally to the level of the trapezoid body rostrally. Every fourth section was silver intensified to visualize the retrogradely transported colloidal gold, borohydride treated and immunohistochemically processed to reveal PNMT-like immunoreactivity. Spinal cord segments were sectioned and silver intensified to localise injection sites.

Briefly, sections were washed 3 × 15 min in 0.1 M phosphate buffer pH 7.2. After 6 × 10 min in 0.1 M sodium citrate/ammonium acetate buffer pH 5.5, sections were incubated in 2 × 20 min changes of IntenSE BL (Janssen). After washing in citrate-acetate buffer the silver processing was stabilised by 5 min incubation in 1% sodium thiosulphate. Sections were rinsed in citrate-acetate buffer, washed 3 × 10 min in 0.1 M phosphate buffer and incubated in 0.5% sodium borohydride in phosphate buffer for 30 min before being rinsed again in phosphate buffer. For immunohistochemistry sections were incubated in 10% normal sheep serum (NSS) in 10 mM Tris phosphate buffered saline pH 7.4 with 0.05% merthiolate (TPBS), containing 0.3% Triton X-100 for 30 min. Sections were incubated in rabbit anti-PNMT serum (now commercially available from Inestar) diluted 1:15000 in TPBS-Triton containing 10% NSS for 48 h. Sections were washed 3 × 20 min in 1% NSS in TPBS-Triton, then incubated in bio-

tinylated sheep anti-rabbit immunoglobulin (Sigma) diluted 1:200 in this buffer containing 1% NSS for 24 h. Sections were washed 3 × 20 min in TPBS-Triton and incubated in 1:1000 Avidin-HRP (Sigma) in TPBS-Triton for 4 h. Sections were rinsed 3 × 10 min in Tris 0.05 M pH 7.6 and immunoreactive cells and fibres were visualised by a peroxidase reaction. Sections were preincubated in 0.5 mg/ml diaminobenzidine in Tris 0.05 M pH 7.6, 0.04% NH₄Cl and 0.2% D-glucose for 10 min and the reaction was started by adding 1 µl of glucose oxidase (Type V-S in 0.1 M sodium acetate buffer, Sigma) per 1 ml of reaction mixture. Reactions were for 20 min, sections were then finally washed 3 × 10 min in phosphate buffer 0.1 M. Sections were mounted on chrome alum slides, dehydrated and mounted in Depex. Using this procedure there was complete antibody penetration and immunoreactivity was observed throughout the 50-µm thick sections. Preabsorption of this antisera with purified bovine PNMT [2], or the substitution of normal for immune serum, resulted in the complete absence of specific neuronal staining in the medulla.

In the reacted sections all PNMT-IR neurons and all PNMT-IR neurons containing retrogradely transported gold (PNMT-IR/CTB-gold-labelled) were counted on both sides of the medulla. After unilateral tracer injection cell counts were recorded ipsi- and contralateral to the spinal cord injection site. Cell counts were not adjusted to whole brainstem numbers. The rostro-caudal locations of PNMT-IR neurons are given in reference to the obex, defined as the rostral extent of the area postrema in the midline. Cell counts were made independently by two observers, and the counts varied by less than 2%. Statistical analysis was by chi-square test using 2 × 2 tables, or by Fisher’s exact test when one of the groups for analysis contained less than five neurons. Results are given as means ± SEM.

**Results**

**Injection sites**

CTB-gold injection sites were localised in the white matter adjacent to the lateral horn spread-
Fig. 1. Distribution of all PNMT-IR neurons and PNMT-IR neurons retrogradely labelled with CTB-gold after bilateral CTB-gold injection into the thoracic spinal cord at levels T2–T4 (n = 4, left) or T8–T9 (n = 4, right). Results are expressed as counts of cells from every fourth 50-μm thick section through the medulla. The distribution of cells rostrocaudally from the obex is given in mm. The distribution of cells in the groups C1, C2 and C3 is described in the text.

Injections sites were less than 0.5 mm in diameter.

**PNMT-IR cell profiles**

PNMT-IR neurons, with amber-stained cytoplasm and showing considerable dendritic detail, were observed over a 3-mm extent of medulla. The rostro-caudal distribution of the PNMT-IR neurons is shown in Fig. 1. Medullary PNMT-IR neurons in the examined series of sections (every fourth 50 μm thick section) from each rat numbered 959 ± 44 (n = 15). These PNMT-IR cells were localised in three cell groups, the ventrolateral C1 group 686 ± 31 cells, the dorsal C2 group 130 ± 10 cells and the dorsomedial C3 group 143 ± 8 cells; respectively 72%, 13% and 15% of the total PNMT-IR neuron population.

The PNMT-IR neurons of the C1 group were located in the ventrolateral medulla in the area of the nucleus paragigantocellularis as defined by Andrezik [4]. Passing rostrally the PNMT-IR cell numbers in the C1 group increased from approximately 1.4 mm caudal to obex in a steady almost linear pattern, to their highest density of 75–85 cells/section at 1.2–1.4 mm rostral to obex. From this level the cell numbers in the nucleus declined over another 1 mm, so that at the caudal edge of the facial nucleus few C1 PNMT cells were to be found (Figs. 1, 3). Throughout this group the C1 neurons were fusiform or multipolar with extensive dendritic branches.
The C2 PNMT-IR neurons extended from the middle of the area postrema caudally to a level approximately 2 mm rostral to obex. The number of cells per section in the C2 group was relatively constant rostro-caudally (Fig. 1). Caudally these cells were small and round, and were located in the dorsal strip area and the dorsal subnucleus of the nucleus of the solitary tract (NTS), and in the commissural subnucleus of NTS. At the rostral levels of the area postrema some larger PNMT-IR neurons appear in the medial portions of NTS. Further rostral these larger neurons were located in the medial and dorsal areas of the NTS and the dorsal motor nucleus of the vagus. These neurons appeared bipolar, with dendrites extending laterally into the NTS and medially towards the midline.

The PNMT-IR neurons in the C3 group extended rostrally from approximately 1 mm to 2.6 mm rostral to obex in the medial longitudinal fasciculus and dorsal to this structure, close to the ventricular surface. Other cells were seen lateral to the fasciculus in the dorsal medullary reticular formation and extending laterally in the prepositus hypoglossal nucleus to the NTS. PNMT-IR neurons in these regions extended rostrally beyond the nucleus tractus solitarius. The C3 PNMT-IR neurons were large multipolar cells, with dendrites extending laterally and dorsoventrally.

Spinally projecting PNMT-IR neurons

PNMT-IR neurons retrogradely labelled from T2–T4

Seven rats were injected with retrograde tracer at levels T2–T4 of the spinal cord. Retrogradely filled PNMT-IR neurons were observed in the areas of the C1, C2 and C3 groups. These neurons contained dense, black particles with an amber-stained cytoplasm. The CTB-gold particles enlarged by silver deposit were visible in the soma and in proximal dendrites (Fig. 2). Other CTB-gold-labelled neurons in the regions of the C1, C2 and C3 groups were not themselves PNMT-IR. CTB-gold-labelled cells were also found in areas of medulla devoid of PNMT-IR neurons — mainly in the ventral midline area and extending laterally to the edges of the pyramidal tracts. In rats injected with tracer at the T2–T4 levels, 920 ± 69 (n = 7) PNMT-IR neurons were counted. 195 ± 16 (21%) of these PNMT-IR cells were retrogradely labelled (Fig. 1). The retrogradely labelled cells, determined as the proportion of each PNMT-IR cell group, comprised 26 ± 2.2% of the C1, 9 ± 1.6% of the C2 and 33 ± 5.1% of the C3 groups, after bilateral injection of CTB-gold (n = 4). The PNMT-IR input to the T2–T4 levels of spinal cord derived largely (76%) from the C1 group, with 4% of input arising from the C2 group and 20% from the C3 group.

Retrogradely labelled PNMT-IR C1 neurons were observed rostrally from the level of the obex (Figs. 1, 2A). The numbers of these CTB-gold-labelled PNMT-IR cells per section increased to a maximum at approximately 1.4 mm rostral to obex, where they comprised 40–50% of the total C1 PNMT-IR population. Further rostral the CTB-gold-labelled PNMT-IR neurons accounted for an increasing proportion of the total PNMT-IR neurons. Virtually all of the most rostrally positioned PNMT-IR C1 neurons were retrogradely labelled. In the C2 group the small PNMT-IR neurons in the dorsal NTS were not retrogradely labelled after spinal cord tracer injections. Only a small population of C2 PNMT-IR neurons were retrogradely labelled and these cells were found medially in the rostral NTS, where the NTS is separated laterally from the ventricle by the prepositus hypoglossal nucleus (Figs. 1, 2B). Some of the most rostrally lying PNMT-IR cells in the C2 group were CTB-gold-labelled. CTB-gold-labelled neurons were found throughout the extent of the C3 group of PNMT-IR neurons (Fig. 1). These CTB-gold-labelled PNMT-IR cells were found scattered in the medial longitudinal fasciculus, some neurons lying adjacent to the ventricle and in the reticular formation (Fig. 2C).

After unilateral injection of tracer at levels T2–T4 a stronger ipsilateral projection from the C1 cells was revealed, with 22 ± 3.2% (n = 3) of PNMT-IR cells of the ipsilateral C1 nucleus being retrogradely labelled compared with 14 ± 0.9% (n = 3) of the contralateral C1 cell group ($X^2 = 6.535$, $P = 0.0106$, Table I). The C2 and C3 cell
Fig. 2. Photomicrographs of PNMT-IR neurons in rat medulla localised in C1 (A), C2 (B) and C3 (C) areas. Higher power photomicrographs on the right side of the boxed areas show PNMT-IR/CTB-gold-labelled neurons (single arrowheads). A. C1 neurons in RVLM at 1.4 mm rostral to obex; CTB-gold injection was at the T4 level; in the low-power field there are 50 PNMT-IR neurons, of which 26 are CTB-gold-labelled. B. C2 neurons in the rostral NTS at 1.0 mm rostral to obex; CTB-gold injection was at the T8 level; in the low-power field there are six PNMT-IR neurons, one of which is CTB-gold-labelled. Three C3 neurons lie close to the ventricle. C. C3 neurons in the medial longitudinal fasciculus and prepositus hypoglossal nucleus at 1.6 mm rostral to the obex; CTB-gold injection was at the T8 level; in the low-power field there are 27 PNMT-IR neurons, 12 of which are CTB-gold labelled.

Calibration bar: low power 100 μm; high power, 30 μm.
groups did not reveal any lateral bias in their projection (Table I).

**PNMT-IR neurons retrogradely labelled from T8–T9**

Eight rats were injected with CTB-gold at levels T8–T9 of the spinal cord. Retrogradely labelled cells were found over a similar rostrocaudal extent of the medulla as observed after T2–T4 level injections (Fig. 1), and in similar medullary regions (Fig. 3). In these rats 993 ± 57 (n = 8) PNMT-IR cells were counted, 170 ± 9 (17%) of these PNMT-IR cells were retrogradely labelled — a proportion similar to that observed after tracer injection in upper thoracic (T2–T4) spinal cord. These CTB-gold-labelled PNMT-IR neurons displayed a similar rostrocaudal distribution to retrogradely labelled PNMT-IR neurons after tracer injection in T2–T4, CTB-gold-labelled PNMT-IR cells comprised 16 ± 1.0% of the C1, 9 ± 3.1% of the C2 and 30 ± 0.8% of the C3 groups after bilateral injection of CTB-gold (n = 4, Fig. 1), so that to the T8–T9 level of spinal cord the PNMT-IR input derived 65% from the C1, 7% from the C2 and 28% from the C3 groups. A significantly lower proportion of the C1 PNMT-IR neurons were labelled from the T8–T9 level than from the T2–T4 level (16% compared to 26% \( X^2 = 16.011, P = 0.0001, \) Table I). The numbers of spinally projecting C2 and C3 PNMT-IR neurons was similar after either T2–T4 or T8–T9 level tracer injections.

After unilateral injection of tracer at T8–T9 levels there were no differences in the two sides of the medulla in the proportions of retrogradely labelled PNMT-IR cells in the C1, C2 or C3 groups.

**Discussion**

The present study demonstrates that in addition to the established projection of the C1 PNMT-IR neurons of the RVLM to the intermediolateral cell column of the thoracic spinal cord, significant numbers of PNMT-IR neurons belonging to the C2 and C3 groups and localised in the dorsal medulla, also project to this region.

Fig. 3. The distribution of PNMT-IR neurons (○) and PNMT-IR/CTB-gold-labelled (●) neurons in one rat injected with CTB-gold bilaterally in the thoracic spinal cord at the T8 level. Each eighth 50-μm thick section through the medulla is presented. Distance is given in mm from the obex.
Investigators have long sought evidence of direct connections of PNMT-IR fibres to the sympathetic preganglionic neurons in order to establish anatomical evidence of a direct neural pathway which might modulate sympathetic outflow and blood pressure. In the light of the present findings it cannot be assumed that PNMT-IR fibres synapsing on identified sympathetic preganglionic neurons [43] arise only in the RVLM as originally suggested by Ross et al. [49]. Some PNMT-IR fibres in this area could arise from dorsal medullary regions — from either the C2 neurons of the NTS-dorsal vagal motor nucleus complex, or the C3 neurons in the medial longitudinal fasciculus.

The topographical distribution of PNMT-IR cells in medulla has been found to be similar to that described previously [30,32,35]. The C1 cell group in the RVLM is the largest of the three medullary PNMT-IR groups of cells, PNMT-IR neurons being more numerous in the C1 group (72% of total medullary PNMT-IR neurons), and also more extensively distributed rostrocaudally.

**PNMT-IR projections to the spinal cord**

A population of PNMT-IR neurons extending rostrally from the obex to the nucleus of the facial nerve projects to the spinal cord. Most of the spinally projecting PNMT-IR neurons are localised in the rostral portions of the C1 nucleus of RVLM, supporting other retrograde transport studies [39,49,50,59] and electrophysiological studies [26]. Approximately 65% (after T8–T9 tracer injection) and 76% (after T2–T4 tracer injection) of retrogradely labelled PNMT-IR neurons were localised within the C1 cell group. This result is similar to the findings of a less detailed study [54], in which absolute cell numbers were not presented, but where 69% of the retrogradely labelled PNMT-IR neuron population were reported to be localised in C1 after large T1–T2 spinal cord injections of the fluorescent tracer true blue. In the present study the proportion of C1 PNMT-IR neurons retrogradely labelled from the upper thoracic cord (T2–T4) was greater than from the lower thoracic cord (T8–T9), suggesting that the adrenergic innervation of the spinal cord is topographically organised. Additionally, the projection of C1 PNMT-IR neurons to upper thoracic spinal cord displayed an ipsilateral predominance, but the projection to the lower thoracic spinal cord was comprised equally of PNMT-IR neurons on either side of the medulla. It is not yet known, however, whether any C1 PNMT-IR neurons project both to upper and lower thoracic levels. Some PNMT-IR neurons in the C1 area also project to

**TABLE I**

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<tr>
<th>Tracer injection in spinal cord</th>
<th>PNMT cell group</th>
<th>Bilateral tracer</th>
<th>Unilateral tracer</th>
<th>Contralateral tracer</th>
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<tr>
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<td>PNMT-IR/CTB-gold</td>
<td>PNMT-IR</td>
<td>PNMT-IR/CTB-gold</td>
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<tr>
<td>T2–T4</td>
<td>C1</td>
<td>585±54</td>
<td>148±16</td>
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<td></td>
<td>C2</td>
<td>109±16</td>
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<td>C3</td>
<td>112±20</td>
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<td>(n=4)</td>
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<tr>
<td>T8–T9</td>
<td>C1</td>
<td>706±50</td>
<td>115±9*</td>
<td>368±36</td>
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<tr>
<td></td>
<td>C2</td>
<td>159±24</td>
<td>12±2</td>
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<td>C3</td>
<td>163±9</td>
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Results are means ± SEM. Number of rats is shown in parentheses. Analysis by $X^2$ test: * $P=0.0001$, comparing numbers of PNMT-IR/CTB-gold cells in C1 after tracer into T2–T4 vs. T8–T9; ** $P=0.0106$, comparing ipsi- vs. contralateral distribution of PNMT-IR/CTB-gold neurons in C1 after unilateral tracer in T2–T4.
higher brain centres [26,47,53,54,55,59]. Anatomical studies have suggested the Cl PNMT-IR neurons projecting to hypothalamus belong almost completely to a population of neurons independent of the spinally projecting Cl PNMT-IR neurons [59], although electrophysiologically-identified, spinally projecting neurons in the Cl region, which are thought to correspond to the PNMT-IR cells, have been found to have collaterals ascending through the central tegmental tract [26]. Possibly these collaterals might innervate the locus coeruleus [47].

Other PNMT-IR neurons projecting to the spinal cord were found in the dorsal medulla. These neurons were more numerous in the medial C3 group than in the C2 group. In contrast with the Cl spinal projection there were no topographical differences in the extent of innervation by the dorsal medullary neurons of the different thoracic levels of the spinal cord. Furthermore, the PNMT-IR innervation of the intermediolateral cell column from the dorsal medulla derived equally from ipsi- and contralateral PNMT-IR neurons. Although spinally projecting neurons from dorsal medulla have been observed previously [1,9,49,52], only incidental observations had suggested that some of these neurons might be adrenergic. Lorenz et al. [39] in investigations of spinally projecting neurons from the RVLM, observed a small number of spinally projecting PNMT/substance P-IR cells along the medial edge of the NTS. Similarly, Hancock et al. noted spinally projecting PNMT-IR neurons in the Cl, C2 and C3 groups [24,25,46]. In the more recent study of Sawchenko and Bohn [54], approximately 31% of the total number of PNMT-IR cells that could be retrogradely labelled following spinal injections have been observed localised in the C2 and C3 regions, a pattern of retrograde labelling similar to that demonstrated in this quantitative study.

Possible role of adrenergic spinal projections in cardiovascular control

The anatomical location of PNMT-IR fibres in the intermediolateral cell column has suggested a role for adrenergic neurons in the control of sympathetic spinal outflow. Projections of medullary PNMT-IR neurons observed in this study to upper and lower thoracic spinal cord are consistent with a cardiovascular role, since in the area of T2–T4 lie some of the cardiac and pulmonary sympathetic preganglionic neurons [57], as well as sympathetic preganglionic neurons projecting in the cervical sympathetic trunk. Lower thoracic cord (T8–T9) contains the highest numbers of sympathetic preganglionic neurons innervating the adrenal medulla (e.g. [3,7]).

The involvement of the rostral Cl PNMT-IR region in mediating changes in sympathetic activity is well documented. Stimulation in this Cl region increases sympathetic nerve activity [16,40,48], circulating adrenaline levels [40,52] and blood pressure [16,21,28,40,44,48,50,52,61]. Furthermore, inhibition of the Cl region of the RVLM evokes a profound fall in arterial pressure and a reduction in basal sympathetic discharge [17]. The observation that spinally projecting presumed PNMT-IR neurons are barosensitive and pulse-modulated [26,45], suggests that the physiological responses are at least in part mediated by adrenergic neurons in this region. That the PNMT-IR neurons in the Cl group might have a role in the reflex control of blood pressure is supported by anatomical evidence of a substantial direct input from the intermediate NTS, an area of baroreceptor afferent input [14], to the PNMT-IR Cl neurons [24].

The dorsal medullary PNMT-IR neurons projecting to spinal cord are also well located to have a role in the regulation of blood pressure, and some evidence suggests that a component of basal sympathetic nerve discharge originates in these areas of the dorsal medulla [8]. It is possible these spinally projecting C2 PNMT-IR neurons might directly mediate baro- or chemoreceptor reflex activity since these neurons are localised in the NTS, the termination site of the baroreceptor afferents [14,34,60]. Furthermore substance P, a putative neurotransmitter in afferent fibres of the IXth and Xth cranial nerves transmitting baro- and chemoreceptor information [20,27], has been observed in processes close to these bulbospinal C2 PNMT-IR neurons [46]. In addition, local circuits within NTS might also mediate activity of PNMT-IR neurons of the C2 group, since the intermediate NTS also targets these neurons [24].
A cardiovascular regulatory role of the C3 PNMT-IR is consistent with the observations of Chai and co-workers [13,37], that chemical stimulation in the rostral dorsomedial medulla near the NTS and in the lateral tegmental field, elevated arterial pressure, possibly by the direct activation of spinoally projecting neurons. The region of the C3 PNMT-IR neurons, the prepositus hypoglossal nucleus, together with the C1 region of RVLM, is a major source of afferents to the locus coeruleus [6] and some of this input arises from the PNMT-IR neurons [47]. It has been observed that discharge of the locus coeruleus parallels sympathetic nerve activity [18]. Such co-regulation of locus coeruleus and sympathetic preganglionic neurons might arise, in the light of present observations, from the neurons of the C3 area in the dorsal medulla and not only from RVLM neurons as previously suggested [6]. Such an association of locus coeruleus function with sympathetic activity, and therefore blood pressure regulation, is consistent with the postulated role of the locus coeruleus in arousal and anxiety mediating systems.

In addition to the observed increases in sympathetic activity that accompany stimulation of the PNMT-IR neuron areas, support for a sympathoexcitatory neurotransmitter role of adrenaline comes also from in vitro studies. Intracellular recordings from sympathet i preganglionic neurons have shown an increase in neuronal excitability and a potential for repetitive discharge, after application of adrenaline [62]. Other studies have however suggested a sympathoinhibitory role for adrenaline, since iontophoresis of adrenaline onto sympathetic preganglionic neurons inhibited their discharge [15,22]. PNMT-IR synapses have now been demonstrated on sympathetic preganglionic neurons, both on perikarya and dendrites [43], and so it is possible that these observed excitatory or inhibitory effects of adrenaline reflect different sites of release of adrenaline onto the sympathetic preganglionic neuron.

In summary, the quantitative evaluation of PNMT-containing neuronal projections from the medulla to the intermediolateral cell column area of thoracic spinal cord suggests a significant role for the dorsal adrenergic cell groups. All the adrenergic neurons are well situated in the brainstem to subserve a sympathetic regulatory function.

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